

## Biodiesel Production from Freshwater Algae

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A novel approach in the production of renewable energy carrier—namely, biodiesel—was performed using algal biomass as a raw material. The effect of drying algal biomass and its role on lipid content during extraction process was also investigated. Transesterification of algal oil was conducted, using ethanol in the presence of potassium hydroxide as a catalyst. A gas chromatography–mass spectroscopy (GC-MS) chromatogram was used to analyze the organic compounds present in the crude biodiesel sample after the transesterification process. The lipid content in the algal biomass was determined to be  $45\% \pm 4\%$ . Biodiesel derived from algae had a fuel value with the following characteristics: density, 0.801 kg/L; ash content, 0.21%; flash point, 98 °C; pour point,  $-14$  °C; cetane number, 52; minimum gross calorific value, 40 MJ/kg; and water content, 0.02 vol %. Copper strip corrosion showed a value less than that of Class 1, which was close to light orange, when compared to the polished strip (i.e., slight tarnish).

### 1. Introduction

The environmental concerns—scarcity in fossil fuel, coupled with government policy—recently have catalyzed the rapid growth of nonfossil transportation fuels. As renewable energy plays a vital role in partially replacing conventional fossil fuel, biodiesel has been become increasingly important in catering the global fuel market. For quite a long period, the production of hydrogen, methane, vegetable oils (triglycerides, for biodiesel), hydrocarbons, and ethanol from algae were in focus as potential biofuels. Although the anaerobic digestion of algal biomass remains as a viable option for methane generation, the advantage of liquid transportation fuels overrides the gaseous form of fuel. Algae has long been an outsider among the known biofuel crops, even though it had several advantages, such as being a potentially greener fuel feedstock, producing more fuel per farmed area of land, and can be grown on nonarable land.<sup>1–3</sup>

Biodiesel is an alkyl esters of long-chain fatty acids obtained from plant seeds (rapeseed oil, soybean oil, palm oil, and sunflower seed) or animal tissues. However, booming biodiesel production has put food and traditional oleochemical industry sectors in threat. Therefore, it is apparently essential to develop nonconventional technology for the production of

triacylglycerides. Lipids can also be produced using oleaginous microorganisms through fermentation.<sup>4,5</sup> Molds, yeasts, and algae also exhibited the capability to assimilate carbohydrates and to accumulate intracellular lipids as high as over 70% (w/w).<sup>6</sup> The potential of algae in the production of biofuel and long-chain polyunsaturated fatty acids (PUFAs) was determined to be promising.<sup>7</sup> The tubular photobioreactors was preferred, in comparison to raceway pond for algal production, because the former resulted in greater productivity with a higher algal oil yield.<sup>8</sup>

Algae are rich in oil and can grow extremely rapidly by doubling their biomass within 24 h; however, in the exponential growth phase, the doubling time is  $\sim 3.5$  h. Oil content in microalgae can exceed 80% by weight of dry biomass.<sup>9,10</sup> The presence of lipids, hydrocarbons, and other complex oils is dependent on the algal species.<sup>11–13</sup> The effect of iron on the growth and lipid accumulation in marine microalgae *Chlorella vulgaris* resulted in an increase in cell density but did not induce lipid accumulation in the late growth phase. While the total lipid content in cultures of late-exponential growth phase when supplemented with  $1.2 \times 10^{-5}$  mol/L  $\text{FeCl}_3$  resulted in an increase in biomass by dry weight (56.6%), which was

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3-fold to 7-fold higher than in F/2–Si medium supplemented with a lower iron concentration.<sup>14</sup>

Biodiesel production from the heterotrophic cells of *Chlorella protothecoides* was performed by extracting the cells in *n*-hexane, followed by acidic transesterification. The optimum process condition occurred at 100% catalyst quantity (based on oil weight) with a molar ratio of methanol to oil of 56:1 at a temperature of 30 °C, which reduced product specific gravity from an initial value of 0.91 to a final value of 0.86 within ~4 h of reaction time.<sup>15</sup> *Schizochytrium limacinum*, when grown on glycerol as a substrate, resulted in a docosahexaenoic acid (DHA, 22:6 n-3) with a yield of 75–100 g/L.<sup>16</sup> The *Schizochytrium limacinum* algal biomass comprised of 45%–50% lipid, 14%–20% protein, 25% carbohydrate, and 8%–13% ash and was rich in lysine and cysteine, while palmitic acid (C16:0) and DHA were the major components in the biodiesel.<sup>17</sup>

The neutral lipid content in microalgae was investigated using the solvent dimethyl sulfoxide (DMSO) as the stain carrier at an elevated temperature in a fluorescence spectrophotometer at an excitation wavelength of 530 nm and an emission wavelength of 575 nm.<sup>18</sup> The effect of lipid production from freshwater microalgae *Chlorella vulgaris* was investigated based on the effect of CO<sub>2</sub> concentration, nitrogen depletion, harvesting time, and extraction.<sup>19</sup> Lipids, when fermented, yielded 65% (w/w) cellular lipid and 100 g/L biomass.<sup>20</sup> The tuber of *Helianthus tuberosus* and the shell of shrimp had also been proved as a feedstock for triacylglycerides.<sup>21</sup> Biodiesel production from lignocellulosic materials was conducted in three stages, namely: (1) hydrolysis of lignocellulose to carbohydrates; (2) fermentation with oleaginous microbes; and (3) transesterification of microbial oil to biodiesel.<sup>22</sup> The role of medium composition (C/N ratio) on *Trichosporon fermentans*, with respect to biomass and lipid production, was determined to be 28.1 g/L and 62.4%. The lipid was comprised of palmitic acid, stearic acid, oleic acid, and linoleic acid, with 64% unsaturated fatty acids.<sup>23</sup>

Biodiesel produced from marine fish soapstock resulted in 37.07 wt % saturated fatty acids, 37.3 wt % long-chain fatty acids, with oleic acid (C18:1) and palmitic acid (C16:0).<sup>24</sup>

Supercritical fluid (SCF) techniques in biodiesel production were reviewed by Wen et al.<sup>25</sup> While the yield of biodiesel with respect to molar ratio, moisture, reaction temperature, kinetics and the characteristics of its emission were reviewed by Sharma et al.<sup>26</sup> The biodiesel produced from the seed of the karanja tree, based on acid and alkaline esterification with NaOH and KOH as a catalyst, resulted in a yield of 89.5% at molar ratios of 8:1 and 9:1 for acid and alkaline esterification, respectively, in the presence of 0.5 wt % catalyst (NaOH/KOH). NaOH was preferred when compared with KOH in terms of yield, as the former resulted in oil with a molecular weight of 892.7.<sup>27</sup> The major components present in biodiesel were analyzed using ultraperformance liquid chromatography in combination with evaporative light scattering detection (UPLC-ELSD). The results showed that oleic acid, methyl cis-9-octadecenoate, linoleic acid, methyl linolenate, stearic acid, methyl octadecanoate, methyl linoleate, palmitic acid, methyl hexadecanoate, erucic acid, and myristic acid as the major component.<sup>28</sup>

## 2. Methods and Materials

**2.1. Collection of Algal Sample.** The algal sample in this present investigation was collected from the outlet channel of Pondy Reservoir, Chennai. Algal biomass was preserved in a rectangular plastic container in open sunlight for a maximum period of 5 days. The container had dimensions of 80 cm length × 50 cm width with a liquid depth of 50 cm. During the algal storage period, the content was mixed for 15 min at a regular interval of once in every 2 h. The air supply was supplied at a rate of 1.5 m<sup>3</sup>/h, during the photoperiod by a diffused aeration system.

**2.2. Analytical Methods.** The total lipids were determined by extracting the algae with chloroform/methanol (2/1, v/v) and was quantified gravimetrically as percent lipid (on a dry weight basis).<sup>29</sup> The potential of algal biodiesel as an alternate for diesel fuel was investigated with respect to density, ash, cloud point, flash point, pour point, cetane number, gross calorific value, water content, and copper strip corrosion, based on the ASTM procedures. GC-MS was used to analyze the organic compounds present in the crude biodiesel sample after the transesterification process.

**2.3. Sequence in Extracting Crude Algal Oil.** The biodiesel production from algae was performed based on the following sequence: (a) air drying, followed by extraction and transesterification; (b) air drying, then soaking the air-dried algae in hexane as a solvent, followed by extraction and transesterification.

**2.4. Production of the Biodiesel from Freshwater Algae.** **2.4.1. Effect of Drying and Expression on Algal Biomass.**

The harvested algae were dried for various periods before subjecting them to expression. The drying period was varied over a range of 0–240 min, and samples were drawn at regular intervals of every 30 min. At the end of the drying

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period, suitable aliquots of sample were expressed and its extractable lipid content was monitored.

**2.4.2. Effect of Soaking Air-Dried Algal Biomass in Hexane Prior to Expression.** The algal biomass, which was dried for a period of 0–240 min in steps of 30 min, was subsequently soaked in a hexane medium prior to the expression process for a period of 30–300 min. On average,  $255 \pm 7$  g of the dried algal biomass was soaked in 50 mL of hexane as a solvent at a constant agitation of 25 rpm. The algal biomass soaked in the hexane was subjected to the expression process at the interval of 30 min and its lipid content was monitored.

**2.4.3. Extraction.** The crude extract obtained from the expression process was a mixture of lipids, water, and traces of algal biomass. The extraction of lipids from the algal liquid was performed using hexane as a solvent in a separating funnel. During the extraction process, the triglycerides enter into the solvent layer, which was decanted and preserved for further processing. The triglyceride that was present in the hexane layer was more viscous. The residual algal biomass was subjected to a further extraction of triglycerides with the addition of hexane. The aforementioned process was conducted 3–4 times, to achieve a higher yield of extractable lipids from the expressed algal liquid.

**2.4.4. Transesterification.** The transesterification process was performed by treating the triglycerides extracted in hexane with ethanol in the presence of potassium hydroxide as a catalyst. During the transesterification process, samples were taken at regular intervals of 30 min, and the reaction was stopped by the addition of water, to determine the extent of the transesterification process. The reaction period was maintained within the range of 30–540 min.

**2.4.5. Fuel Properties of Algal Biodiesel.** The characteristics of algal biodiesel were evaluated with respect to the following properties: acid number, calorific value, cetane number, flash point, ash content, and pour point. GC-MS peaks were also analyzed to determine the constituents present in the crude algal biodiesel.

### 3. Results and Discussion

**3.1. Drying and Expression of Algal Biomass.** The air drying of algal biomass was performed using a sieve (sieving mesh) that had a clear opening of 5 mm in a shady area in the absence of direct sunlight. A suitable aliquot of air-dried algal biomass was removed from the mesh, on a volume basis, in the form of squares (which averaged to a weight of  $255 \pm 7$  g). The sample was subjected to expression using a mechanical expresser. During the expression process, the amount of lipids expressed from the algal biomass is as shown in Figure 1. For example, at a drying period of 30 min, the lipid content in the expressed liquid was determined to be 38%. For a drying period of 90 and 120 min, the lipid content was 46% and 48%, respectively; however, a drying period of 240 min resulted in a lipid content of 34%. The reason for low lipid content at 30 min was the presence of excess water in the expressed algal liquid. At drying periods of 90 and 120 min, the lipid content in the expressed algal liquid showed a maximum value. Further increasing the drying period up to 240 min resulted in a lower yield in the lipid content, because the algal biomass was overdried. Therefore, the optimum drying period was determined to be 120 min. Hence, further experiments were performed by air-drying the sample for 120 min.

**3.2. Soaking of Dried Algal Biomass in Hexane Prior to Expression.** The air-dried algal biomass, as stated in the

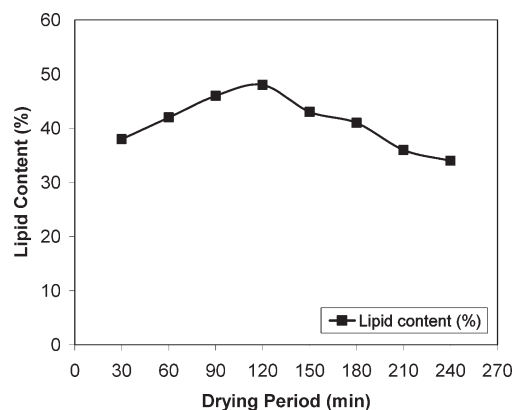


Figure 1. Lipid content versus algal drying period.

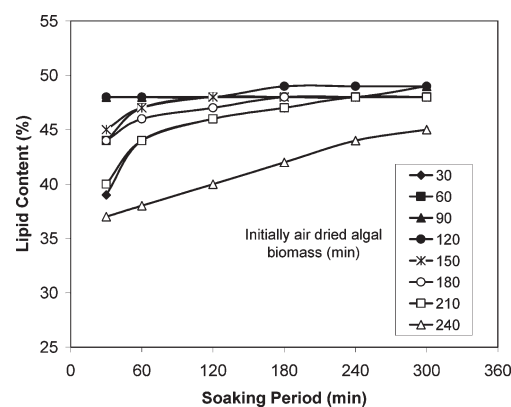


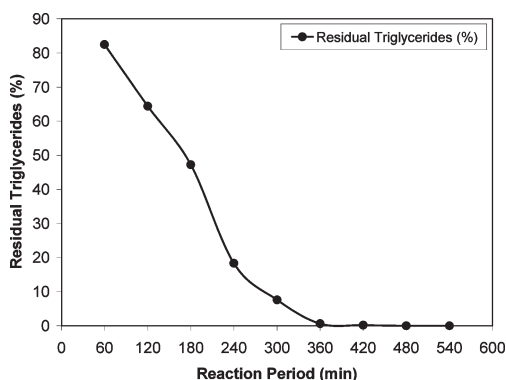
Figure 2. Lipid content versus soaking period in hexane (air-dried algal biomass).

drying process, was subjected to soaking in the hexane for various time periods, viz, 30–300 min. The results showed that soaking followed by expression helped to increase the extraction of lipids from algal biomass as shown in Figure 2. The algal biomass, which was dried initially for a period of 30 min, was subjected to soaking in hexane for a period of 30, 180, and 300 min, resulted in a lipid content of 40%, 47%, and 48%, respectively. However, an initial algal air-drying period of 120 min, followed by soaking in hexane for 30, 180, and 300 min, resulted in a lipid content of 48%, 48%, and 49%, respectively. In the case of an algal drying period of 240 min, followed by soaking in hexane for the aforementioned time period, resulted in a lipid content of 37%, 44%, and 47%, respectively. A gradual increase in lipid content of the expressed liquid was observed as the soaking period in hexane was increased, which could be due to softening of the dried algal biomass by the solvent action.

**3.3. Extraction.** Hexane was used as a solvent for the extraction of triglycerides from the crude liquid extract of algal biomass. To attain a higher yield of triglycerides, the hexane was added in excess (as high as 2.5 times of that of the crude extract, on a volume basis). At a hexane to crude algal oil ratio of less than 1:1.5, more of the crude lipid oil was determined to be sticking on the container, thereby leading to lower yield. Hence, the volume of crude algal extract to that of hexane was maintained at a ratio of 1:2.5.

**3.4. Transesterification.** The objective of the reaction is to complete the transesterification reaction process, thereby resulting in biodiesel that was low in triglyceride concentration. In the transesterification process, an excess of alcohol and catalyst





**Figure 3.** Residual triglycerides versus the transesterification reaction period.

(KOH) was used to ensure maximum transesterification. The transesterification process was conducted at the following ratio (250:75), on a volume basis of triglyceride:ethanol, with 1.25% potassium hydroxide as a catalyst. During the process, the triglycerides are converted into fatty acid ethyl esters. The completeness of reaction was ascertained by drawing the samples at different time intervals and measuring the triglycerides content, as shown in Figure 3. Following the transesterification process, the biodiesel was washed with water, to remove the glycerol from the fuel.

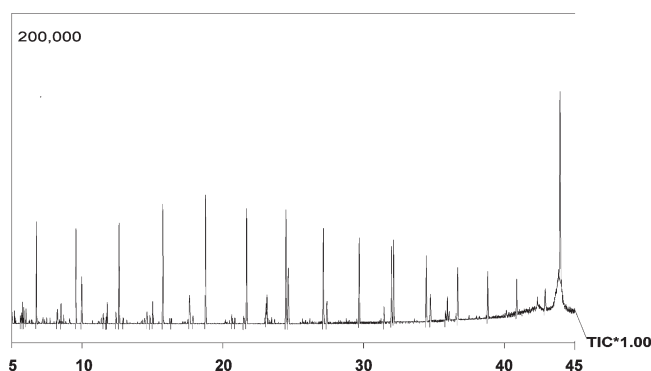
**3.5. Fuel Properties of Algal Biodiesel.** The acid number of algal biomass in this present investigation was determined to be 0.4 mg KOH/g. The acid numbers for algal oil<sup>30</sup> and microalgae *Chlorella protothecoides*<sup>31</sup> were determined to be 0.71 and 0.37 mg KOH/g, respectively. The biodiesel produced from the *Brassica carinata* plant<sup>32</sup> and waste cooking oil<sup>24</sup> resulted in acid values of 0.12 and 0.69 mg KOH/g, respectively. Frying oil yielded a higher acid value of 3 mg KOH/g, because of the presence of 1 wt % of water content in the raw oil.<sup>33</sup> Similarly, a high acid number of 1.17 mg KOH/g was observed in marine fish oil biodiesel.<sup>24</sup> The moisture hydrolyzes the esters present in the biodiesel into alcohol and acids, thereby leading to higher acid numbers.

The calorific value of the fuel determines the fuel consumption required to obtain the same energy power output. The biodiesel derived from the algal oil resulted in a value of 40 MJ/kg, whereas a corroborating energy value of 41 MJ/kg was stated by Xu et al.<sup>31</sup> when algae served as a substrate. The calorific value of biodiesel produced from marine fish oil and waste cooking oil was determined to be 41.37 and 40.11 MJ/kg, respectively.<sup>24</sup> A calorific value that was 12.7%–14.7% lower than the ASTM value was observed by Monyem and Van Gerpen.<sup>34</sup>

The cetane number indicates the quality of the compression ignition of diesel fuel. A larger cetane number for a

**Table 1.** Characteristics of Biodiesel Fuel Value Derived from Algal Biomass in Comparison with Indian Standard Biodiesel

parameter	Value	
	algal biodiesel	Indian Standard
density	801 kg/m <sup>3</sup>	860–900 kg/m <sup>3</sup>
ash	0.21 mass %	0.01% mass %
flash point	98 °C	120 °C
pour point	–14 °C	less than –10 °C
calorific value	40 MJ/kg	37.27 MJ/kg
cetane number	52 min.	51 min.
acid number	0.4 mg KOH/g	0.50 mg KOH/g
water content	<0.02 vol %	<0.03% vol %
copper strip corrosion	≤Class 1 (slight tarnish)	Class 1



**Figure 4.** GC-MS chromatogram of crude biodiesel obtained from algal oil after transesterification.

diesel fuel indicates the shorter ignition delay and duration of the combustion period, less occurrence of knocking, and lower emission of nitrogen oxides.<sup>35</sup> Graboski and McCormick<sup>36</sup> found that the cetane number of biodiesel produced from soybean oil was in the range of 45.7–56.4 min. The cetane number of the biodiesel derived from algal oil in this present investigation resulted in a value of 52 min, whereas biodiesel from marine fish oil, waste cooking oil and corn oil had a value of 50.9, 48.1, and 49 min, respectively. ASTM standard for the No. 2D diesel was determined to be 53.2 min in the case of cetane number.<sup>24</sup>

Algal biodiesel produced in this present investigation from freshwater algae resulted in a flash point of 98 °C, whereas Xu et al.<sup>31</sup> stated a value of 115 °C with *Chlorella protothecoides*. The biodiesel derived from the corn oil,<sup>30</sup> marine fish oil,<sup>24</sup> and waste cooking oils by Chen et al.<sup>37</sup> and Lin and Li<sup>24</sup> resulted in values of 103.9, 103, 195, and 141 °C, respectively. The Germany DIN V51606 and ASTM D6751 biodiesel standards for flash point were determined to be ≥110 and ≥130 °C.<sup>37</sup> The ASTM No. 2D diesel had a flash point of 74 °C, which was much lower than the value stated in the biodiesel. As liquid fuel with a high flash point can prevent auto ignition and fire hazards at high temperatures during transportation and storage periods, the biodiesel derived from algae has an edge over the No. 2D diesel.

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Table 2. GC-MS Peak TIC Data of Crude Biodiesel Obtained from Algal Oil after Transesterification

peak	retention time (min)	area (%)	name
1	5.202	0.35	hexylcyclohexane
2	5.617	0.21	(E)-ethyl 5- <i>N</i> -benzamido-2-pentenoate
3	5.710	0.34	sulfone, butyl isopropyl
4	5.783	0.45	2,2-dimethylbutane
5	5.867	0.44	allyl isobutyrate
6	6.027	0.32	2,2-dimethylbutane
7	6.759	3.47	<i>n</i> -undecane
8	8.254	0.83	<i>tert</i> -butyltrichlorosilane
9	8.514	0.73	1-methylbutyl nitrite
10	9.570	3.74	<i>n</i> -dodecane
11	9.980	2.35	isobutyl disulfide
12	11.509	0.37	1-methylbutyl nitrite
13	11.721	0.28	2-methyl-3-pentanone
14	11.793	0.82	sulfurous acid, isobutyl pentyl ester
15	12.410	0.51	methylnaphthalene
16	12.634	4.32	<i>n</i> -dodecane
17	12.925	0.24	(3 <i>R</i> ,5 <i>R</i> )-3-methyl-5-(2'-phenylethyl)-4-oxahept-6-insaure
18	14.610	0.41	allyl isobutyrate
19	14.820	0.24	2,2-dimethyl-3-hexanone
20	15.021	0.92	<i>n</i> -docosane
21	15.738	5.35	pentadecane
22	16.340	0.22	1,1-dimethyl-1,2-dihydronaphthalene
23	17.634	1.83	<i>n</i> -pentadecane
24	17.888	0.25	<i>tert</i> -butyl methyl-3-oxopentanoate
25	18.769	6.16	pentadecane
26	20.638	0.34	1-methylbutyl nitrite
27	20.845	0.20	4-methyl-3-pentanone
28	21.477	0.37	1-(1-(1-cyclopentanyl)-2-oxocyclopentyl)-4-phenyl-1,2,4-triazolidine-3,5-dione
29	21.692	5.65	pentadecane
30	23.079	0.57	<i>n</i> -docosane
31	23.139	0.62	1,4-benzenedicarboxylic acid, diethyl ester
32	24.482	5.89	2,7,10-trimethyldodecane
33	24.652	3.85	<i>n</i> -tetradecane
34	27.147	4.87	pentadecane
35	27.400	1.61	<i>n</i> -docosane
36	29.689	4.22	<i>n</i> -tetracosane
37	31.451	0.56	eicosyl acetate
38	31.978	3.64	hexadecanoic acid, ethyl ester (CAS) ethyl palmitate
39	32.123	3.87	<i>n</i> -tetracosane
40	34.448	3.23	<i>n</i> -octadecane
41	34.747	1.62	<i>trans</i> -phytol
42	35.814	0.41	5,6-dichlorohexene
43	35.953	1.05	6(E),9(Z),13(E)-peneptriene
44	36.097	0.45	1-Propyne, 3-ethoxy-(CAS) Ether
45	36.680	2.84	<i>n</i> -hexadecane
46	38.817	2.60	<i>n</i> -docosane
47	40.866	1.84	<i>n</i> -docosane
48	42.898	1.24	<i>n</i> -docosane
49	43.952	13.33	bis(2-ethylhexyl) phthalate
total		100.00	

The ash content of algal biodiesel derived from freshwater algae was determined to be 0.21 wt %. Biodiesel produced from marine fish oil, waste cooking oil, and the ASTM No. 2D diesel were determined to be 0.76, 0.26, and 1.57 wt %, respectively.<sup>24</sup> The existence of impurities, ash, and additives in liquid fuel may affect the quantity of carbon residue after the fuel is burned. Thus, burning the algal biodiesel was observed to produce less residual carbon than the biodiesel derived from marine fish oil and waste cooking oil. The density of the biodiesel produced from the freshwater algae had a value of 0.801 kg/L, whereas the heterotrophic microalgae *Chlorella protothecoides* had a value of 0.864 kg/L. The waste cooking oil<sup>37</sup> and *Brassica carinata* plant biodiesel<sup>32</sup> resulted in densities of 0.8924 and 0.810 kg/L, respectively.

The pour point in the present investigation had a value of −14 °C, whereas the microalgae *Chlorella protothecoides* showed a value of −11 °C. The water content in the biodiesel derived from algal oil in this present investigation had a value of 0.02 vol %. The copper corrosion showed a value of less

than that of Class 1, which represented slight tarnish of the copper strip. Table 1 shows the comparison between the characteristics of algal biodiesel and the Indian standard of biodiesel.<sup>38,39</sup>

The relative weight compositions of organic compounds present in the algal biodiesel was analyzed using GC-MS, as shown in Figure 4 and Table 2. The major compounds were determined to be, viz, pentadecane, *n*-docosane, *n*-tetracosane, *n*-dodecane, 2,7,10-trimethyldodecane, *n*-tetradecane, *n*-undecane, *n*-octadecane, and *n*-hexadecane, respectively.

## Conclusion

Experimentation on biodiesel production was conducted using freshwater algae. Although all algae has long been an

(38) Kaul, S. N. *Quality Criteria of Biodiesel*. Asian Publishers: New Delhi, India, 2005.

(39) Satish, L. Indian Biofuels Awareness Centre, <http://www.svlele.com>. (Accessed on August 1, 2009.)

outsider among the known biofuel crops, its potential has been recognized recently, because of the increased yield in algae oil to those of traditional oilseeds. Algae has the potential of growing in places away from the farmlands and forests, thus minimizing the damages to the ecosystem and food chain supply. Moreover, algae can also be grown in sewage and next to power-plant smokestacks, where they can digest the pollutants and deliver the oil. The present investigation had proved the successfulness of implementing the

biodiesel production from algae as a raw material. The algal drying and soaking periods were also optimized to yield algal oil with a higher lipid content. Biodiesel extracted from the freshwater algae was comparable in quality with that of conventional biodiesel. Future research should concentrate in identifying or developing high-lipid-content microalgae or bioengineering microalgae for biofuel production, because it would be the most promising way in catering the energy demand.